

GC-MS analysis of Essential oil of *Pogostemon cablin* Benth. (Patchouli oil) of Nepal

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Abstract

Patchouli oil was extracted from shade dried patchouli leaves by hydro-distillation method using Clevenger distillation apparatus and the oil percentage was 3.5%. Specific gravity, refractive index, optical rotation, acid value and ester value were determined. The quantitative analysis was performed by GCMS. The quantitative analysis showed Patchouli oil contained alpha guaiene (9.95%), seychellene (7.14%), alpha patchoulene (7.08%), alpha bulnesene (13.16%) and patchouli alcohol (32.54%) as major compounds. The aims of this research were to investigate patchouli oil separation by fractional distillation, determination of physico-chemical properties and GC-MS analysis.

Keywords: GC-MS, Patchouli, Physico-chemical parameters

Introduction

Patchouli (*Pogostemon cablin* Benth.) is a commercially important aromatic plant belonging to the family Lamiaceae, which contains patchouli essential oil. The name 'Patchouli' comes from Tamil language which means green leaves. Its oil is commercially used in perfumes and cosmetics (Hasegawa et al., 1992; Maheswari et al., 1993). It also possesses anti-insecticidal, anti-fungal and bacteriostatic properties (Kukreja et al., 1990). The decoction of the leaves is used with other drugs to treat nausea, vomiting, diarrhea, cold and head ache in China (Mohideen, 2006).

It is used in aromatherapy to calm nerve, relief depression and stress. The oil is extensively used as a flavoring ingredient in major food products, including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatin and meat products. The oil is regarded as fixative for heavy perfumes which imparts strength, character, alluring notes and lasting qualities. This oil is highly used in food and perfumery industries and there is no synthetic substitute for patchouli oil and hence it has a great demand in perfumery industries (Guenther, 1990; Corine, 2004).

P. cablin plant grows 400m above sea level. It prefers a warm humid climate with fairly heavy and evenly distributed rainfall of 2500-3000 mm per annum, a temperature of 24° to 28°C and average atmospheric humidity of 75%. In areas, irrigation is needed for its well growth and partially shaded area is best for its vegetative growth. It is relatively a hardy plant and adapts itself to a wide range of soil conditions. A well drained loamy soil rich in humus and nutrients with a loose friable structure and with no impervious hard layer at the bottom is ideal for patchouli cultivation. A pH range of 5.6-6.2 is suitable for its growth. It is shade loving crops so can be grown as an intercrop with other trees. It is widely cultivated and grown in Indonesia, India, Malaysia, Philippines, China, Brazil and Singapore (Farooqui and Sreeramu, 2001).

The quality of patchouli oil is mainly measured by the amount of patchouli alcohol present in it. The minimum amount of patchouli alcohol in high quality patchouli oil is more than 30%. Patchouli oil containing norpatchoulene, gives the smell and aroma that; is typical of patchouli oil. The temperature and time duration of distillation process influence the percentage yield of essential oil (Benjamin, 1995).

The Literature review revealed that typical patchouli oil is extracted by farmers has low level of patchouli alcohol commonly below 30%. This low grade oil consequently produces patchouli oil with low market price. The level of major components in patchouli oil can be increased by appropriate determination of fractional distillation in better temperature and pressure condition (Alisyah and Anwar, 2012). In order to achieve a better yield of patchouli oil at shorter drying time, mechanical drying of the herbage is good option (Ambrose and et al., 2013)

The earlier study reported that Indonesia plays a significant role, about 90% of world demand met by Indonesia. The components of Indonesian patchouli are beta- patchoulene (2.9-3.8%), alpha-guaiene (12.1-15.2%), Caryophyllene (3.3-3.9%), alpha- patchoulene (5.1-5.9%), alpha-bulnesene (4.7-16.8%) and patchouli alcohol (32-33.1%) (Muyassaroh et al, 2016). The Specified quality parameter requirements of Patchouli oil of Indonesia National Standard (Harunsyah and M. Yunas, 2012) is shown in table:

Materials and Methods

Extraction of Essential Oil (EO)

Fresh leaves of Patchouli (*P. cablin*) was collected in Dec. 2015 from Jhapa District located in Western Development Region of Nepal. Pre-treatment such as drying, withering and size reduction was

performed to obtain optimal results. This experiment was conducted in the Natural Products Research Laboratory; at Department of Plant Resources, from January to August 2016. The collected fresh leaves were shade dried for 70 days and 100 g air dried leaves were hydro distilled in a Clevenger apparatus for 8 hours. The essential oil was thus obtained was dried over anhydrous sodium sulphate, filtered and stored in a sealed glass vial at 4°C prior to the analysis.

Primary reference standard of patchoulol (patchouli alcohol) with AM 0795 batch HWI01638 (1 ml) manufactured by HWI Analytik GMBH pharma solution Ruelzheim, Germany was purchased having patchouli alcohol amount of 291.72 mg/g.

Physico-chemical parameters

For determination of the physiochemical parameters following standard methods were performed:

Thin layer Chromatography (TLC) analysis

The oil was monitored by TLC in solvent system toluene: ethyl acetate (93:7) and Anisaldehyde methanolic sulfuric acid (0.5 ml Anisaldehyde + 10 ml glacial acetic acid + 85 ml methanol+ 5 ml conc. sulfuric acid) was used as developing reagent. The spot were visualized in day light as well as under UV light at 254nm and 366nm. TLC foils percoated silica gel 60 GF254, 0.2mm were of Merck, Darmstadt, Germany.

Table 1: Specified standard quality requirements of patchouli oil

Parameter	Standard Requirements	Parameter	Standard Requirements
Color	Light yellow- Reddish brown	Acid Value	Maximum 8 to 0
Odour	Hidden fruit woody aroma	Ester Value	Maximum 20 to 0
Refractive index	1.507 to 1.515	Patchouli Alcohol	Minimum 30%
Optical Rotation	(-)48° to (-)65°	Specific gravity 25°C	0.950 to 0.975

Table 2: Methods Employed For Physicochemical Parameters

Parameter	Method
Oil Percentage	Hydro-distillation of dried leaves using Clevenger apparatus, British Pharmacopia, Vol. 11.1988 (Appendix XI E A137E volatile oil in Drug)
Specific Gravity (Density)	AOAC 19 th Edition, 2012 (Vol. II Ch-41, Page 2-3 method no. 985.19)
Refractive Index	ISO 280:1999 (E)
Optical Rotation	AOAC 19 th Edition, 2012 (Vol. II Ch-36, Page 19-20method no. 920.142)
Acid Value	ISO 1242:1999 (E)
Ester Value	British Standard methods of tests for essential oils (1953)

Quantitative analysis of essential oil

The Patchouli alcohol was analyzed by Gas Chromatograph Mass Spectrometer (GCMS) and content of patchouli alcohol in patchouli oil is compared to meet the requirements of National standard. A GCMS 2010 (Shimadzu Co., Japan) system with an RTx-5MS column (30m × 0.25mm i.d., 0.255Ø film thickness) was used for the analysis. The injector temperature was adjusted at 200 °C. Helium was used as a carrier gas at a constant flow rate. The MS parameters were adjusted as ion source and interface temperature at 200 °C and 250 °C respectively. The detector voltage was set at 0.70 kV; with event time of 0.5 sec and a mass range of 40–550 m/z.

For quantitative analysis, gradient temperature program was set in the oven temperature as shown in the table 3; with carrier gas flow rate of 0.99 mL/min and an injection volume of 2.5 µL was injected under split ratio. For MS parameters, SCAN mode was selected for acquisition with start m/z 2 min and end m/z 18 min. The identification of components of the essential oil was based on comparison of their mass spectra with those stored in NIST library, 2005.

Table 3: Oven Temperature Program for Quantitative Analysis

Temperature gradient (0°C/min)	Temperature (°C)	Hold Time (min)
-	70	0
15	100	2
2	160	2
20	250	6

For quantitative analysis, oven gradient temperature program was followed as shown in the Table 2; flow rate was 1.49 mL/min, injection volume was 1ml

Table 4: Physico – chemical parameters of patchouli oil sample

S. No.	Parameters	Results
1	Physical state /Color	Liquid/ Dark reddish brown
2	Odor	Distinctive ,woody aroma with hidden fruity odor
3	Solubility	Soluble in alcohol / insoluble in water
4	Oil Percentage	3.5% ± 0.2
5	Specific Gravity (Density)	0.9623 at 20°C
6	Refractive Index	1.512 at 20°C
7	Optical Rotation	26.92° at 16°C
8	Acid Value	0.5929
9	Ester Value	1.7749

with split injection of 1:200. In MS section, SIM mode was selected for acquisition with start m/z 2 min and end m/z 45 min. The fragment ions monitored during analysis were m/z: 222, 138, 125, 98, 55 and 41. For the purpose of quantification of patchouli alcohol (PA) in the sample oil, first reference patchouli oil containing of PA was run in GCMS to analysis and then the patchouli oil Sample was injected with the same method described above.

Results and Discussion

Physico – chemical parameters

The physico-chemical parameters give us the idea of its physical nature and some chemical properties. The Physico – chemical parameters determined were as follows:

Thin Layer Chromatographic (TLC) analysis

Both the reference and sample patchouli oil were loaded separately on the same TLC plate for comparison. Mixture of toluene: ethyl acetate (93:7) and Anisaldehyde methanolic sulfuric acid (0.5 ml Anisaldehyde + 10 ml glacial acetic acid + 85 ml methanol+ 5 ml conc. sulfuric acid) was used as developing reagent. Then the TLC plate was heated in oven at 110 p C for 10 minutes.

Table 5: Major spots observed CO-TLC

S.N.	Spots	Rf values
1	Spot-1	0.14
2	Spot-2	0.29
3	Spot-3	0.50
4	Spot-4	0.59
5	Spot- 5	0.86



Figure 1: TLC of Sample and Reference Patchouli oil

The five major spots were observed during TLC analysis in patchouli oil. And the last spot which is large compared to others must contain highest percentage in given oil.

GCMS analysis

The chromatogram of GCMS analysis is shown in fig. 2 below:

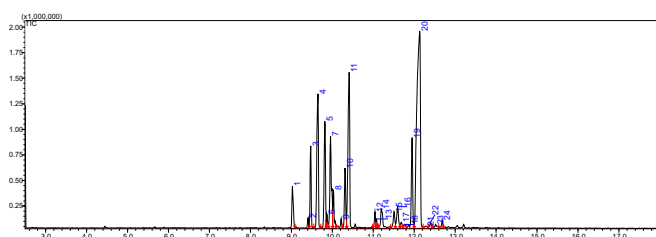


Figure 2: Chromatogram of Patchouli alcohol sample

The GCMS data analysis gives the chemical composition of the patchouli oil sample along with identification and tentative percentage composition of the constituents present in the oil sample. The following table 6 shows the number of constituents identified and their relative percentage in the patchouli oil.

The above table 6 shows chemical composition of our patchouli oil and a total of 16 chemicals were identified. It also indicates that the five major constituents have highest area % which are Guaiene (9.95%), Seychellene (7.14%), Patchoulene (7.08%), Bulnesene (13.16%) and Patchouli alcohol (32.54). This was also indicated by the five large spots of CO-TLC analysis. Tentatively, Patchouli alcohol was the major chemical that shows maximum % composition i.e. 32.54%.

Table 6: Chemical compounds present in the patchouli oil sample identified by GCMS

S.No.	Name of Chemical Constituents	Retention Time (min)	Area % (MS)
1	Beta-Patchoulene	9.03	2.88
2	Seychellene	9.41	0.56
3	Caryophyllene	9.47	4.37
4	Alpha-Guaiene	9.65	9.95
5	Seychellene	9.82	7.14
6	Alpha Humulene	9.87	0.69
7	Alpha-Patchoulene	9.96	7.08
8	Aromadendrene	10.03	2.05
9	Selinene	10.31	3.18
10	Bulnesene	10.41	13.16
11	Spathulenol	11.51	1.35
12	Palustrol	11.60	1.95
13	Kessane	11.68	0.47
14	Globulol	11.76	0.49
15	Viridiflorol	11.95	5.89
16	Patchouli alcohol	12.13	32.54
	Total		100

Conclusion

Experiment concluded that the essential oil of Patchouli leaves can be extracted by simple hydro distillation method and further the chemical composition and other physico-chemical parameters were also studied which gives the idea about the physical nature and chemical compounds present in *P. cablin*. The major chemical compounds from GCMS analysis were beta patchoulene (2.88%), caryophyllene (4.37%), alpha-Guaiene (9.95%), seychellene (7.14%), alpha-patchoulene (7.08%), aromadendrene (2.05%), beta Selinene(3.67%), alpha-bulnesene (13.16%), spathulenol (1.35%), patustrol (1.95%), viridiflorol (5.89%) and patchouli alcohol (32.54%). The main component of patchouli oil i.e. patchouli alcohol is found to be 32.54% in our oil which can contribute to a high trade value of the oil in international market.

Acknowledgements

The authors are grateful and like to offer profound gratitude to the DDG Mrs. Susma Upadhyaya and Mr Sanjeev Kumar Rai. We offer sincere thanks to Mrs. Jyoti Joshi Bhatta, Chief of NPRL for her great motivation and encouragement and Mr. Rajendra Sharma for his valuable suggestion. We are obliged

to Mr. Tara Dutta Bhatta, Mr. Keshav Paudel, Mr. Anjani Kumar Adhikari, Mr. Krishna Kumar Shah, Mr. Rajeswar Ranjitkar, Mr. Madan Raj Bhatta, Mr. Bhawani Prasad Adhikari and all the staff of NPRL and DPR for their co-operation and team work.

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